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JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

Contents

A Comparison of the Bromthymol Blue Milk Test and the Methylene Blue Reduction Test for Determining Quality of Milk. E. D. DEVEREUX..... 367

A Survey of Present Methods of Grading Milk at Condenseries, Evaporated and Dry Milk Plants..... 374

A Color Defect of Process Cheese. WALTER V. PRICE..... 377

Copper in Dairy Products and Its Solution under Various Conditions. II. J. MISCALL, G. W. CAVANAUGH AND P. P. CARDEMOS..... 379

An Unappreciated but Important Factor in the Pasteurization of Milk. J. M. SHERMAN, C. N. STARK AND PAULINE STARK.. 385

Further Studies of the Rôle of Vitamin C in the Nutrition of Calves. L. M. THURSTON, L. S. PALMER AND C. H. ECKLES..... 394

The Solubility of Calcium Phosphate in Fresh Milk. E. O. WHITTIER..... 405

Effect of Cottonseed Meal and Linseed Oil Meal on the Consistency of Feces of Dairy Cattle. C. F. HUFFMAN AND L. A. MOORE. 410

Book Reviews..... 419

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Cloth, 6 x 9, ix + 78 pages, illustrated, bibliography.

LABORATORY MANUAL OF FIELD ECOLOGY by V. E. SHELFORD $10.00
Cloth, 6 x 9, xii + 608 pages, 219 figures, 56 tables, bibliography, index.

THE CHILD'S HEREDITY by PAUL POPENOE $2.00
Cloth, 5¼ x 8½, xiii + 316 pages, cover design, 27 illustrations, bibliography, index.

ORDINALL OF ALCHIMY by THOMAS NORTON $2.50
Leather back, marble boards, paper label, 5½ x 8½, viii + 126 pages, illustrated with wood cuts.

POPULAR RESEARCH NARRATIVES VOL. III edited by A. D. FLINN $1.00
Cloth, 5 x 7½, viii + 174 pages, illustrated, index.

FORTHCOMING

CORONARY THROMBOSIS: ITS VARIOUS CLINICAL FEATURES by SAMUEL A. LEVINE, to be published Autumn 1929, probable price $3.00.

THE DOCTOR IN COURT by EDWARD HUNTINGTON WILLIAMS, to be published about September 21, probable price $3.00.

SOIL SCIENCE INDEX, VOLS. I–XXV, edited by JACOB G. LIPMAN, to be published about September 20, probable price $3.00.

THE MOST NEARLY PERFECT FOOD by SAMUEL J. CRUMBINE AND JAMES A. TOBEY, to be published about September 15, probable price $2.50.

BLOOD GROUPING IN RELATION TO CLINICAL AND LEGAL MEDICINE by LAURENCE H. SNYDER, to be published about October, 19, probable price $5.00.

YARDSTICK OF PUBLIC UTILITY OPERATIONS AND CONSTRUCTION COSTS by WM. W. HANDY, to be published about October 1, probable price $10.00.

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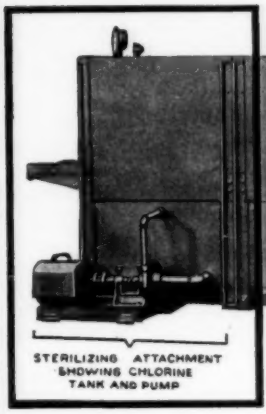

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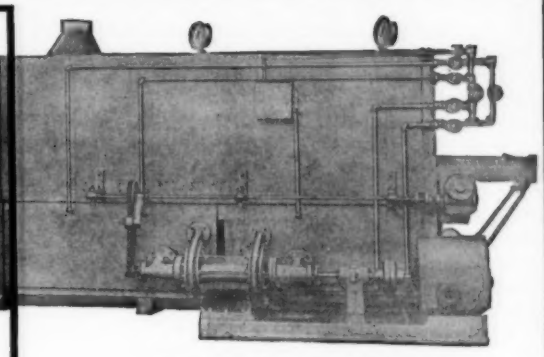

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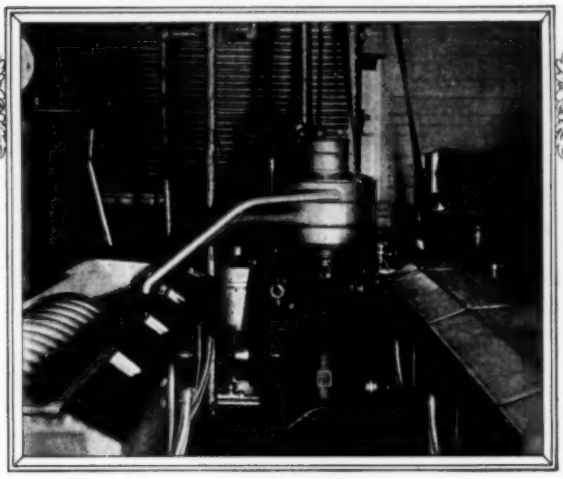

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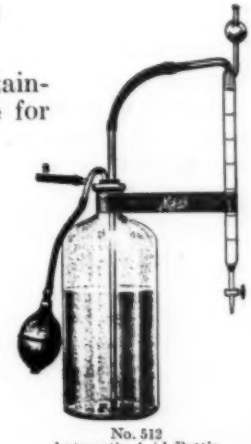

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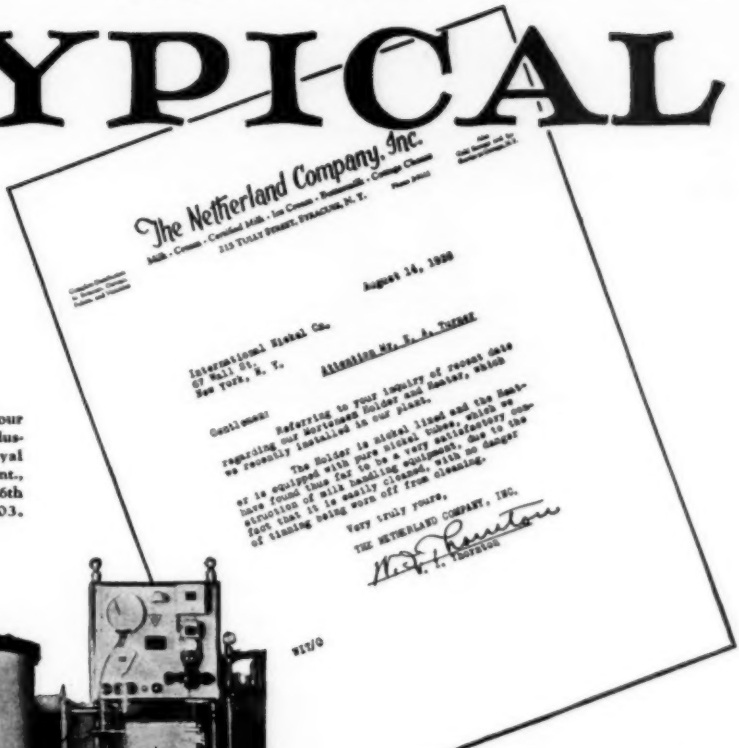
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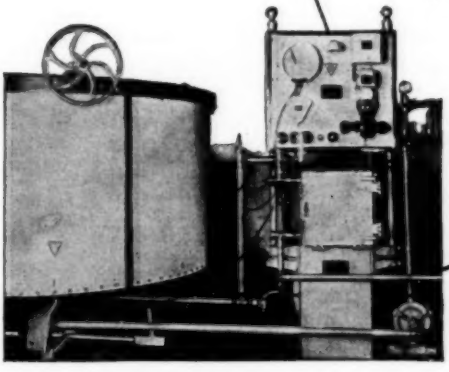

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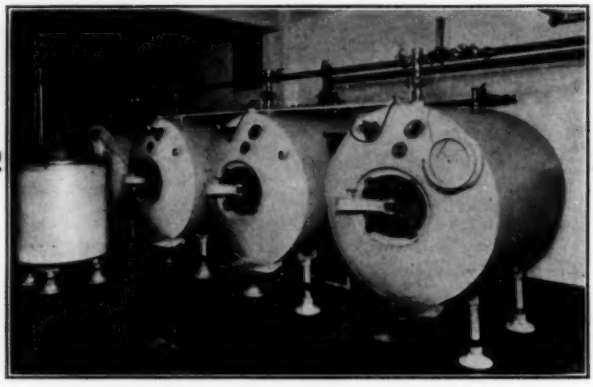

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UNBIASED DATA AGAIN POINT TO GLASS AS MOST SUITABLE FOR DAIRY EQUIPMENT

New investigation conducted at University of California analyzes effect of various metals on milk. See July issue of the Milk Dealer.

Again confirming the findings of many other authorities, a new investigation, carried on under the direction of Messrs. E. S. Guthrie, C. L. Roadhouse, and G. A. Richardson, in the Division of Dairy Industry, University of California, points out the effects of various metals on milk. It is again significant to observe that glass-lined equipment remained inert to the action of milk, whereas practically every type of metal showed some effect on its flavor. Pfaudler glass-lined equipment assures natural flavor and maximum purity, whether the milk is pasteurized or stored at low temperature. See July issue of "The Milk Dealer," article entitled "Determines the Effect of Milk on Metals and Glass."

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A COMPARISON OF THE BROMTHYMOL BLUE MILK TEST AND THE METHYLENE BLUE REDUCTION TEST FOR DETERMINING QUALITY OF MILK*

E. D. DEVEREUX

Michigan Agricultural Experiment Station, East Lansing, Michigan

REVIEW OF THE LITERATURE

The bromthymol blue milk test or colorimetric hydrogen ion method was devised by Cooledge (1, 2) in 1920 to determine the keeping quality of milk. The test was compared to other methods, particularly the methylene blue reduction test and the standard plate count. These comparisons were made by using correlation coefficients. In each case the correlation coefficient between the test in question and the keeping quality was determined; the results were as follows:

Bromthymol blue score (Cooledge score)	+0.75
Methylene blue reduction test	+0.52
Agar plate count	−0.25

Cooledge measured the keeping quality by the time required for the milk to show the first appearance of souring, as determined by taste. The milk was held at room temperature. One hundred fifteen samples were examined. The bromthymol blue scores were made according to the method as devised by Cooledge (see experimental); the methylene blue reduction test was applied as described by Hastings (3); the plate counts were made according to "Standard Methods" (1919).

Ellenberger et al. (5) made a comparison of the methylene blue reduction test and the agar plate count for determining the quality of milk. The keeping quality (time) of 268 samples was determined. The correlation coefficients were as follows:

Methylene blue reduction test	+0.72	±0.02
Agar plate count	−0.28	±0.04

* Received for publication January 11, 1929. Paper No. 3 (n.s.) from the Michigan State College Agricultural Experiment Station.

Ellenberger et al. measured the keeping quality by the time required for the milk to become unfit for table use as determined by taste and acid development. The bacteriological examinations were made according to "Standard Methods."

EXPERIMENTAL

To the knowledge of the author the studies of Cooledge have never been repeated, and, since the work of Ellenberger, et al. on the reduction test did not corroborate the results secured by Cooledge, it was desired by the author to repeat some of this work.

In the experimental work the bromthymol blue test and the methylene blue reduction test were compared, using the actual keeping quality (time) as the standard.

One hundred fifty 200-cc. samples were taken in 250-cc. sterile Erlenmeyer flasks from the receiving vats of two dairies.

The bromthymol blue test was performed as devised by Cooledge with the exception that a slight change (6) has been made in the formula of the Cooledge broth. The test is made by adding 0.1 cc. of the milk in question to 10 cc. of bromthymol blue broth and noting the rate of pH change when incubated at 37°C. until a pH of 5.8 is reached or until the tube has been incubated for eight hours.

Formula of Cooledge broth[1]

Beef extract	3 grams
Peptone	10 grams
Sodium chloride	5 grams
Dibromthymosulphonephthalein (Bromthymol blue)	0.020[2] grams
Distilled water	1000 cc.

Adjusted to pH = 7. Autoclaved at 15 pounds for fifteen minutes.

Bromthymol blue color standards[3] having a pH range of 5.8 to 7.4 (5.8, 6.0, 6.2,. . . , 7.4) are used for making the readings.

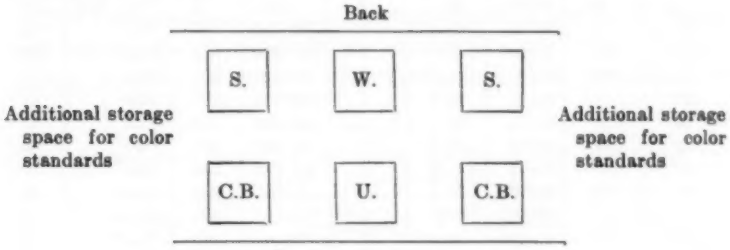
[1] This is the formula that has been recommended to the Digestive Ferments Company, Detroit.

[2] Recently changed from 0.032 to 0.020 gram per liter.

[3] The test has been so devised that the color standards and comparators of either the Hynson, Westcott, Dunning Company, (use test tubes with a diameter of 1.8 cm.) or the LaMotte Chemical products Company, can be employed (use tubes with a diameter of 1.5 cm.).

Broth without the indicator is also prepared and used in the compensating blanks. The compensating blanks are inoculated and incubated in the same manner as the broth tubes containing the indicator. It is necessary to prepare only two compensating blanks for one day's run or any one batch of samples.

When it is desired to make a reading, the tubes and the compensating blanks are removed from the incubator, cooled to 20°C., and placed in the comparator. The top view of the arrangement of the tubes in the comparator when a reading is to be made is given below.



S. = color standard
W. = water blank
C.B. = compensating blank
U. = unknown containing the milk in question

The unknown is compared to the color standards and the pH noted.

Cooledge devised a table in order that the milk could be scored, its keeping quality determined, and the purpose for which it is best suited determined. From experience with this table the author is of the opinion that milk scoring 70 or better is suitable for milk plant supply

The methylene blue reduction test was made according to "Standard Methods" (fifth edition, 1927), using approved standardized tablets from which a fresh solution was prepared every few days.

The keeping quality was measured by the time required for the

milk to become unfit for table use as determined by taste. The samples were held at 18° to 20°C.

TABLE 1

The comparison of the results secured by the bromthymol blue and methylene blue tests and the keeping quality of milk

TIME OF SOURING	NUMBER OF SAMPLES	BROMTHYMOL BLUE TEST			METHYLENE BLUE REDUCTION TEST			
		Score range*	Average score	Number of exceptions	Reduction range†	Average reduction time	Milk class†	Number of exceptions
hours					*minutes*	*minutes*		
8	2	35–55	37.5	0	0–20	3	Very bad	0
12	9	35–55	44.4	0	20–120	57	Bad	3
18	31	35–55	50.2	2	120–330	123	Fair	18
20	16	35–55	51.2	2	120–330	228	Fair	7
22	8	60–75	58.7	8	120–330	180	Fair	5
24	8	60–75	59.4	8	120–330	218	Fair	4
26	14	60–75	65.0	12	120–330	317	Fair	4
28	13	60–75	71.0	9	120–330	289	Fair	6
30	13	60–75	70.5	12	330+	342	Good	8
32	14	80–100	85.0	2	330+	424	Good	3
34	10	80–100	85.0	2	330+	413	Good	2
36	8	80–100	89.4	2	330+	466	Good	2
38	2	80–100	90.0	0	330+	585	Good	0
44	2	80–100	95.0	0	330+	525	Good	0
	150			59				62

* Score range:

READING	pH	SCORE	MILK SUITED FOR
hours			
1, 2, 3	5.8	20–30	Condemning
4, 5, 6, 7, 8	5.8	35–55	For butter making
8	5.9–6.2	60–75	Condensing
8	6.3–6.7	80–100	Market milk or cheese making

† "Standard Methods" classification of milk according to reduction time:
Class 1—good milk, not decolorized in 5.5 hours
Class 2—fair milk, decolorized in 2 to 5.5 hours
Class 3—bad milk, decolorized in 20 minutes to 2 hours
Class 4—very bad milk, decolorized in less than 20 minutes

In table 1 the results are arranged according to the time required for the samples to become unfit for use. Since souring

was, in the great majority of the cases, the first undesirable quality displayed, the column is headed "Time of souring."

An examination of the table shows that as the time of souring increased there was a steady increase, with two exceptions, in the bromthymol blue average scores. In one of the exceptions the score dropped 0.5 of a point as the time of souring increased two hours, while in the other case the average score remained stationary. The average reduction times show more fluctuations than the other scores, but it will be noted that, if these average reduction times are interpreted in terms of "milk classes," there is a steady increase in the classes as the time of souring increases.

The average score or reduction time for each group of samples determined the score range or reduction time range for that group, as given in table 1 with footnotes. This permitted the

TABLE 2

The correlation coefficients of the colorimetric tests and the keeping quality of milk secured by three investigators

METHOD	COOLEDGE'S COEFFICIENTS	ELLENBERBER'S (ET AL.) COEFFICIENT	THIS AUTHOR'S COEFFICIENTS
Bromthymol blue test.....	+0.75	No experimental work	+0.77
Reduction test.....	+0.52	+0.72 ±0.02	+0.77

detection of those samples whose score or reduction time did not fall in the determined range. These cases were counted and the number in each group recorded under the column heading "Number of exceptions."

Comparing the two tests from the standpoint of exceptions both seem to have about the same accuracies in detecting the poor milk and the good milk, and about the same inaccuracies in regard to the intermediate grade.

The individual cases, that is the score and reduction time of each sample (data too voluminous to present), were used to calculate the correlation coefficients between the two methods and the keeping quality of milk, and, strange to say, were both found to be +0.77. This corroborates the work of Cooledge on the bromthymol blue test but not his work on the reduction test.

However, Ellenberger et al., as mentioned earlier, found the reduction test to have a correlation coefficient of $+0.72 \pm 0.02$ which is in accordance with the coefficient found for that method in this work. These results of the three investigations are summarized in table 2.

In reviewing the technique used by Cooledge in performing the reduction test it was found that he used one part of dry dye to 100,000 parts of milk, which is about twice the concentration of the dye when the approved tablets are used. The reduction time of methylene blue of this concentration was determined on 75 samples of milk and compared to the reduction time when the approved tablets were used. In every case when methylene blue of a concentration of one part of dry dye to 100,000 parts of milk was used the reduction time was from a few minutes to forty minutes longer than when a solution of an approved tablet was used. This may account for some of the difference in the results since the approved tablets were used in this work and the work of Ellenberger et al. However, according to Hastings (4) it should not be a great factor. He found that the methylene blue could be reduced to 1:300,000 or 1:400,000 parts of milk without materially changing the reduction time.

SUMMARY

The bromthymol blue milk test was found to fulfill its purpose in that it graded milk fairly accurately into a large number of classes according to keeping quality, when average scores were studied.

The reduction test was found to fluctuate more but was able to grade the milk into four classes equally as accurately.

The correlation coefficients between each test and the keeping quality were both found to be $+0.77$.

The writer wishes to express at this time his gratitude to Prof. L. C. Emmons of the Mathematics Department of Michigan State College for his assistance in interpreting these data.

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(2) Cooledge, L. H. 1921 The colorimetric hydrogen ion determination as a means of studying biological changes in dairy products. Michigan Agr. Exp. Sta., Tech. Bul., No. 52.

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A SURVEY OF PRESENT METHODS OF GRADING MILK AT CONDENSERIES, EVAPORATED AND DRY MILK PLANTS

The need of definite information regarding the methods in use at the present time in this branch of the dairy industry is evident to the sub-committee on Bacteriological Methods for Condensed, Evaporated and Dried Milk of the American Dairy Science Association. By making use of mailing lists furnished by the American Dry Milk Institute and Evaporated Milk Associations addresses of the companies manufacturing the products were obtained. Through the suggestion of Dr. R. S. Breed, Chairman of the General Committee, an effort was first made to get in touch with the person in each organization that was directly in charge of the control work for each particular company. Thirty-one companies expressed their willingness to coöperate and were sent a questionnaire asking for information on the subject. From the 31 companies 20 questionnaires have been received up to the present time. Thirteen companies were manufacturing condensed, the same number evaporated, 12 spray powder, 4 roller process powder and 3 reported that they manufactured powder but failed to specify the kind manufactured.

The reports regarding the examinations of the milk as received indicate that the conventional "nose" test is still used on each can at the majority of the plants. Questionable milk is further checked by a variety of tests. Acidity determinations are used by 12 plants to check such milk, 5 used the alcohol coagulation test, while 1 used the alcohol cresol purple test, 1 the brom cresol purple test, another the alcohol methylene blue test and 1 the live steam test. The use of the sediment test was reported in 11 cases and was used at irregular intervals.

The methylene blue reductase test was used by 5 companies and one reported that it gave unsatisfactory results. Seven plants used the breed microscopic count and 3 the plate count, all of this work being done in a more or less irregular and unstandardized way.

The standards used for rejecting milk were, acidity 0.18 to 0.20 per cent, temperature over 60° to 65°F. and coagulation with alcohol test. The bacterial standards varied from 100,000 to 1,000,000 per cubic centimeter, and milk showing reduction of methylene blue in less than two and one-half hours was considered unsatisfactory.

In reporting methods of sterilizing equipment 10 used some form of hypochlorites or chlorine solution, 5 used steam, 1 hot water, 1 cleaning agents and 3 failed to report. Seven stated that they checked the sterility of the equipment. The methods used for checking sterility varied greatly. Three reported the use of sterile water rinse, 1 used the count obtained from the water run through, 1 used the count from the first milk through, while 2 failed to specify method used. The standards used varied with the method used.

In checking the finished product, 7 companies indicated that a regular check was made by the plate method. One of these also ran the American Public Health Association test for *B. coli* as given in the Standard Methods of Water Analysis, 2 reported the occasional use of the plate method and methylene blue test, 1 used incubation tests on canned goods only, and 10 failed to report. Two companies, however, stated that they were installing bacteriological laboratories in the near future.

The standards for the finished product as reported by 6 companies were as follows: all required evaporated milk to be sterile, 2 required condensed milk to have a count under 1,000 colonies per gram, 1 below 10,000, 2 below 25,000 and 1 under 100,000. Two companies reporting on milk powder required a count of less than 300,000 and 500,000 per gram.

In general it would seem that from these results that the bacteriological control of the manufacture of condensed, evaporated and powdered milk is not as well established as it should be and that there is a need for information regarding practical methods of such control. It is the hope of the committee that they may secure sufficient coöperation to be able to draft a report that will fill this need. Such a report should be of value to the industry in encouraging better control of these products.

Perhaps the most important need at the present time, is a standardization of the method of determining the count from the finished product, especially milk powders. There is a prospect that a joint study of methods for this purpose may be carried out during the coming year with the Committee on Laboratory Methods appointed by the American Dry Milk Institute.

P. A. DOWNS, *Chairman*,
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Lincoln, Nebraska, June 2, 1928.

A COLOR DEFECT OF PROCESS CHEESE*

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A manufacturer of a brand of process cheese found that occasional batches developed an undesirable, dark color during the heating process. This color matched very closely the color of an orange with a brownish tinge or that of thymol blue in solutions of pH 2.0 to 2.4. Although the cheese in all other respects seemed to be entirely normal, it was rejected by the trade.

The product was manufactured by grinding, and mixing together, well ripened cheese of the cheddar type, and placing the mass in a steam jacketed kettle. Water, an alkaline solution of annatto color, and an emulsifying agent were added to the cheese, and the mixture was heated slowly with constant agitation. The normal color usually darkened during the last stages of the heating process.

Observations indicated that the color change was independent of the quality of the cheddar cheese used in the blending operations, since identical cheese to which the same ingredients were added did not always develop the defect.

It seemed logical to assume that the change was associated in some manner with the heating operation itself. During this process some of the moisture was lost, the amount of which varied with the temperature used and the duration of the heating period. Laboratory trials were made to determine the influence on the color of the cheese of variations in its moisture content. The results indicated that the color could be slightly deepened by reducing the moisture content of the cheese approximately 2 per cent or more. Other trials showed that the color could be noticeably darkened by raising the temperature and increasing the duration of the heating process. These treatments, however, did not produce the characteristic color defect.

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It was customary in the factory to place a new batch of cheese in the heating unit as soon as the preceding batch was removed. In this way the machine was in continuous use throughout the day, except for the noon recess. As a result, more or less cheese gradually accumulated around the sides and edges of the kettle. As would naturally be expected, this residual cheese assumed a darker color when it was exposed to the heat of subsequent batches. The accumulation of this discolored cheese, and the fact that the defective color usually appeared after the noon hour, when most of this substance adhered to the kettle, strongly suggested that this residual cheese was responsible for the defect.

An attempt was made to reproduce this defect in the laboratory by duplicating the factory routine as nearly as possible. The cheese was allowed to remain on the sides of the kettle in which the blending and pasteurizing was accomplished as it did in factory practice. A quantity of cheese sufficient for several batches was divided into identical portions. Batch after batch was made with no appearance of the defect. The kettle was then allowed to stand idle for two hours and the cheese adhering to it from the previous batches was not removed. The next batch of cheese made in this kettle developed the defective color. A second batch acted in the same manner. The cheese which was used in making the batch was then pasteurized in the usual manner in a clean kettle and was entirely normal in its appearance. The kettle in which the defective cheese was made was then thoroughly cleaned and another portion of the same cheese was pasteurized in it. The cheese retained its normal color. In repeated trials the defect could be reproduced and corrected by following this same procedure. These facts seemed to verify the theory that the incorporation of sufficient amounts of the dried and discolored cheese which accumulated on the unwashed kettle was actually capable of producing the undesirable color.

The manufacturer who originally called this defect to the attention of this laboratory took the necessary precautions to eliminate the possibility of incorporating this residual cheese in new batches. After a two years' interval no repetition of the trouble has occurred.

COPPER IN DAIRY PRODUCTS AND ITS SOLUTION UNDER VARIOUS CONDITIONS. II*

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AND

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This work is a continuation of the investigation conducted by Rice and Miscall.[1]

Quam, Soloman and Hellwig[2] have published data on the copper dissolving power of milk. They find the temperature of maximum copper solubility between 85° and 90°C. They used thirty minutes as the time of exposure of the milk to the metal. They attribute the decrease in the copper solubility curve above 90°C. to the decreasing solubility of oxygen.

METHOD OF PROCEDURE

In this investigation the method of Rice and Miscall[1] was followed. This consisted of allowing 2000 cc. of milk to stand, in glass flasks, in contact with twisted strips of copper, under the conditions of the experiment for two hours. The solution was then poured off the copper and evaporated to dryness, the residue ignited in a muffle furnace and the ash dissolved in a small quantity of nitric acid. The copper was electrolized onto a platinum flag, washed thoroughly with distilled water, and re-electrolized from a weak sulfuric acid solution onto a weighed rotating platinum cathode. By using this method of electrolysis a bright, adherent deposit of pure copper is obtained which can be weighed.

The pasteurized milk used was obtained directly from the

* Received for publication March 4, 1929.

† Presented to the Faculty of Cornell University as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[1] Rice and Miscall, Jour. Dairy Sci., 1923, vi, 261.

[2] Quam, Soloman and Hellwig, Jour. Indust. and Eng. Chem., (1928), xx, 930.

pasteurizing apparatus of the Dairy Department at Cornell. The raw milk was obtained at the Cornell barns.

SOLUBILITY OF COPPER IN PASTEURIZED MILK

Two-liter portions of pasteurized milk were placed in 5-liter flasks. The milk was then heated to the desired temperature. As soon as the correct temperature was reached fourteen twisted, bright copper strips 1 inch wide and 5 inches long (140 square inches of surface) were placed in the flask. The milk was heated at constant temperature for two hours. The copper content of the milk was then determined. The results are tabulated in table 1.

The data are plotted in figure 1 as curve *A*.

This curve shows the same values of copper content and temperature of maximum copper solubility as obtained by Rice and Miscall.[1] The copper solubility increases up to a temperature of 140°F. and then decreases as the temperature is raised. Both increase and decrease in solubility taking place gradually.

SOLUBILITY OF COPPER IN RAW MILK

The previous experiment was repeated except that raw milk was used. The results are given in table 1.

The data are plotted in figure 1 as curve *D*. The same type of curve results as when pasteurized milk was used but the temperature of maximum copper solubility is at 150°F. It is also interesting to note that previously pasteurized milk dissolves more copper at every temperature than does raw milk.

EFFECT OF CARBON DIOXIDE AND OXYGEN ON COPPER SOLUBILITY

To determine the effect of specific gases upon the solubility of copper in milk the following experiments were performed. A 5-liter flask was stoppered with a two holed rubber stopper. Through one hole a glass tube was lead which connected the flask with a manometer. Another glass tube connected the flasks with a mercury filled, gas burette which had a two-way stopcock. The burette could be used to admit known amounts of gas into

the flask and also served as a mercury pump in the usual manner. Two liters of raw milk were placed in the flask.

All the gases were exhausted from the milk, the temperature being raised to the desired value. The pumping was continued until the manometer showed a pressure equal to the vapor pressure

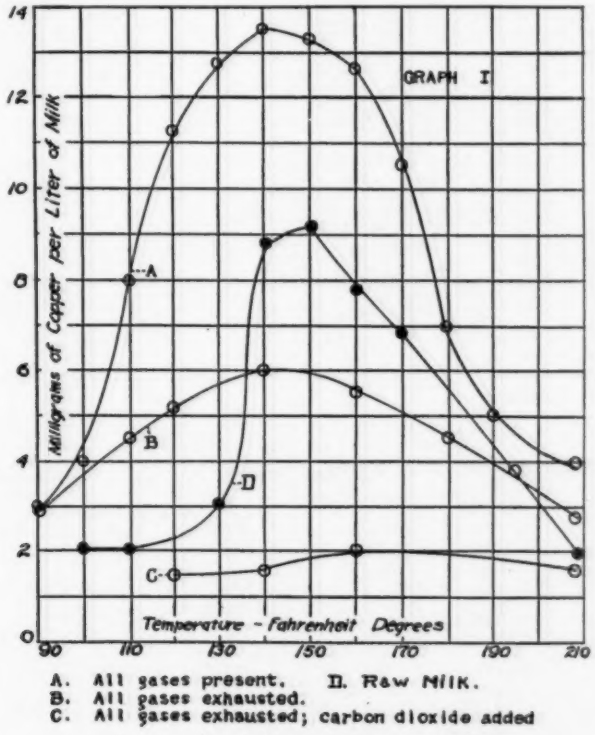

FIG. 1. SOLUBILITY OF COPPER IN MILK

of water at that temperature. Then the stopper in the flask was quickly removed and 14 strips of copper (140 square inches) were introduced. The admitted air was quickly pumped out. The temperature was maintained constant for two hours. Then the milk was analyzed for copper in the usual manner. The results are given in table 2.

The data are plotted as curve *B* in figure 1. It shows a much lower copper solubility than either the raw or pasteurized milk and a maximum copper solubility at 140°F.

TABLE 1

Solubility of copper in pasteurized milk and in raw milk

TEMPERATURE	COPPER PER LITER OF MILK	
	Pasteurized	Raw
°F.	mgm.	mgm.
70*	3.0	
100	4.0	2.0
110	8.0	2.0
120	11.3	2.5
130	12.8	3.2
140	13.5	8.8
150	13.3	9.2
160	12.7	7.8
170	10.5	6.8
180	7.0	
190	5.0	
208†	4.0	2.0

* Room temperature.
† Boiling.

TABLE 2

Solubility of copper in milk from which all gases had been exhausted

TEMPERATURE	COPPER PER LITER OF MILK
°F.	mgm.
70	3.0
100	
110	4.5
120	5.25
140	6.00
160	5.50
180	4.50
190.5	3.75
208	2.75

The same procedure was then followed but after all the gases were exhausted excessive amounts of carbon dioxide were added and the milk heated in contact with the copper open to the atmosphere for two hours. The results are given in table 3.

The data are plotted as curve *C* in figure 1. It shows that carbon dioxide has a definite depressing effect upon the solubility of copper in milk.

The curves show that the solubility of copper in pasteurized milk is greater than in raw milk. Both milks exhibit a maximum copper dissolving power at a definite temperature, this temperature being 5 degrees higher in raw milk than in pasteurized milk. The temperature range of maximum copper solubility is from 140° to 150°F. The copper dissolving power of milk at the boiling temperature is very near to that at room temperature. The presence of carbon dioxide greatly decreases the copper dissolving power of milk. The removal, by exhaustion, of all the gases

TABLE 3

Solubility of copper in milk in which the natural gases had been replaced by carbon dioxide and oxygen

TEMPERATURE	COPPER PER LITER OF MILK	
	Carbon dioxide added	Oxygen added
°F.	mgm.	mgm.
120	1.5	11.00
140	1.55	14.00
160	2.00	13.00
208*	1.60	5.00

* Boiling point.

normally dissolved in the milk also decreases the copper dissolving power of milk. Hence the presence or absence of carbon dioxide does not explain the solubility of copper in milk products.

To test the effect of oxygen the same procedure was followed as in the proceeding experiment except that oxygen was added. The data are given in table 3.

The results obtained from this run show that a maximum dissolving power of milk is obtained even in the presence of oxygen. The results approximate those obtained when pasteurized milk was used but are higher than when raw milk was used. Oxygen therefore cannot account wholly for the copper solubility of milk because raw milk should contain more oxygen than pasteurized

milk and therefore should dissolve more copper, but the converse is true. The fact that a maximum is obtained in the solubility curve, at about the same temperature as is obtained with raw or pasteurized milk, shows that there is a reaction over which oxygen has no control.

CONCLUSIONS

1. The copper dissolving power of milk increases directly up to 140° to 145°F. and then decreases.

2. Either the removal of the milk gases or the addition of carbon dioxide decrease the copper dissolving power of milk.

3. The presence of oxygen increases the amount of copper which goes into solution but does not change the general type of the solubility curve as obtained in the other experiments.

4. Pasteurized milk is shown to dissolve more copper than raw milk at the same temperature.

5. It is concluded that some reaction takes place in the milk serum, when milk is heated for two hours in contact with bright copper, above the temperature of pasteurization, which decreases the copper dissolving power of milk.

AN UNAPPRECIATED BUT IMPORTANT FACTOR IN THE PASTEURIZATION OF MILK*

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That physiological differences exist in bacterial cells of various ages was shown by Sherman and Albus (5). They found that young cells of bacteria are more easily killed by various harmful agents, including heat, than are the old cells of the same organisms. In their paper were suggested a number of practical applications which might be made of these observed physiological differences in bacterial cells. With respect to pasteurization it was stated:

> To cite another example, in an entirely different field of bacteriology the pasteurization of milk may be mentioned. As has been shown by Ayres and Johnson (1913), the efficiency of pasteurization can not be judged, as was formerly thought, on the basis of the percentage of bacteria destroyed. It is found that in the case of milks with high bacterial counts (in which there has necessarily been an extensive growth of bacteria) it is easy to obtain an efficiency above 99 per cent, while with milks of low bacterial count (in which there has been little or no proliferation of cells) no such percentage destruction is obtained. It is of course recognized that this fact is probably due in the main to the differences in the types of bacteria predominating in these classes of milks, but it is possible that the physiological condition of the cells with respect to age also plays some part.

Although the factor of physiological youth in bacteria has not heretofore been considered by workers on pasteurization, a number of other applications have been reported by other investigators. As examples, it has been shown that the age of the bacterial cells dealt with is important in connection with the determination of thermal death rates (Robertson (3)), and (Stark

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and Stark (8)); in studying the germicidal action of milk (Sherman and Curran (6)); in making bacterial plate counts (Albus (1)); in the control of gassy fermentations of Swiss cheese (Rogers (4)); in studies of the rate of fermentation by bacteria (Stark and Stark (8)); and in studies of heat production by bacteria (Bayne-Jones and Rhees (2)).

EXPERIMENTAL

In another paper Stark and Stark (8) have reported the results of a number of experiments dealing with three important milk bacteria, *Streptococcus fecalis*, and two different types of ropy milk organisms. A number of strains of each of these

TABLE 1

Influence of pasteurization on pure cultures of young and mature bacterial cells

TYPE	AGE	BACTERIAL PLATE COUNTS		RATIO BEFORE:AFTER	PER CENT SURVIVAL
		Before pasteurization	After pasteurization		
	hours				
1	24	149,500,000	3,250,000	46:1	2.17
	4	11,050,000	10,900	1,014:1	0.0986
2	54	91,000,000	45,000	2,022:1	0.049
	6	98,000	<1	>98,000:1	<0.001
3	54	21,900,000	6,750	3,244:1	0.0308
	6	485,000	<1	>485,000:1	<0.0002

organisms were studied and in every case it was found that at the temperature of pasteurization (62.8°C. for thirty minutes) the young cells of these organisms were destroyed more rapidly than were the mature cells. In table 1 are reproduced one typical test with each of these organisms. The young cells of *Streptococcus fecalis* were obtained by growing the culture in milk for four hours at 37°C., while old cells were procured from a culture which had been grown for twenty-four hours under the same conditions. In the case of the ropy milk organisms the cells were obtained from six- and fifty-four-hour cultures incubated at laboratory temperature. In each case the culture was trans-

ferred to sterile skimmed milk before subjecting it to the heat test.

The practical significance of the age of the bacterial cells in the pasteurization of milk is plainly evident from these data. Of particular interest are the results obtained with these two types of ropy milk organisms which reveal the fact that they may be entirely eliminated from milk if the cells are in the young and actively growing condition, while the old cells of the same organism may withstand this treatment. It has frequently been stated that the common causes of ropy milk are destroyed by efficient pasteurization, whereas it is now demonstrated that the efficiency of pasteurization for this purpose is dependent upon physiological age of the bacteria. As a matter of fact, the two types of slime producing bacteria used for these experiments were obtained from a milk plant which was experiencing difficulty with ropiness in its pasteurized product.

While there can be little doubt that such pure culture observations as have been cited are of practical importance to the dairy industry, it is desirable to have specific data on normal milk and its mixed bacterial population. It is obvious that if milk after production is kept at a low temperature so that its bacterial content does not increase, the bacteria contained therein will be old. On the other hand, it is equally obvious that if the milk is held at higher temperatures, so that the bacterial population multiplies, the cells of the microörganisms present will be relatively young. This being the case, we would expect to obtain a higher percentage efficiency in the destruction of bacteria in the case of milks in which growth has taken place. In table 2 is presented the result of a simple experiment which shows the validity of this deduction. Two samples of milk, one with a relatively low bacterial content and the other with a relatively high bacterial content, were held for twenty-four hours at 40°F. (4.4°C.). At the end of this period, each sample was divided into two portions, one portion of each being pasteurized at 145°F. (62.8°C.) for thirty minutes, while the other portion was incubated at 37°C. for four hours and then submitted to the pasteurization treatment. Bacterial counts by the plate culture

method were made on all portions immediately before and after pasteurization. The plating medium used in all of these experiments was milk powder agar and the plates were incubated at 37°C. for three days.

From the data given in table 2 it is seen that the proportion of bacteria killed in the samples which were held at 37°C. is much greater than in the same samples which were pasteurized immediately after storage at low temperature. In other words, it is again shown that young bacterial cells are much more sensitive to heat than are old cells; and further, that this physiological difference in bacteria is of practical significance in the pasteurization of milk.

TABLE 2

The effect of the age of bacterial cells in a mixed milk flora on the efficiency of pasteurization

SAMPLE NUMBER	AGE	INCUBATION TEMPERATURE	BACTERIAL PLATE COUNT		RATIO BEFORE: AFTER	PER CENT SURVIVAL
			Before pasteurization	After pasteurization		
	hours	*°C.*				
1	24	4.4	10,100	3,250	3:1	32.17
1-A	4	37	490,000	2,400	205:1	0.489
2	24	4.4	575,000	460	1,250:1	0.08
2-A	4	37	82,000,000	28,500	2,877:1	0.0347

1-A = Sample 1 after being incubated for four hours at 37°C.
2-A = Sample 2 after being incubated for four hours at 37°C.

It is, of course, appreciated that the results thus far reported might be criticized as not being "practical," as it is a rather far cry from 37°C. to the temperatures which are more commonly used for the storage of milk supplies. It remains to be shown, therefore, that this factor of physiological youth in bacteria is an actual one under the conditions attaining in the commercial handling of milk supplies. If the principle holds, we would expect to find a greater percentage efficiency when pasteurization is applied to milk which has been held at 60°F. (15.5°C.) than when applied to fresh milk or milk which has been held at temperatures below the limits of growth of the normal milk bacteria.

In order to test this hypothesis, the following experiments were conducted: Various samples were obtained which represented milks soon after production and which had been cooled so that bacterial growth was prevented. All of these samples were of milks produced under reasonably sanitary conditions. One of the samples (as may be noted from the high bacterial count) was

TABLE 3

Bacterial efficiency of pasteurisation applied to milk when fresh and held at different temperatures

SAMPLE NUMBER	AGE	INCUBATION TEMPERATURE	BACTERIAL PLATE COUNT		RATIO BEFORE: AFTER	PER CENT SURVIVAL
			Before pasteurization	After pasteurization		
	hours	*°C.*				
1	0		3,700	250	15:1	6.75
	24	4.4	4,850	50	97:1	1.03
	24	15.5	149,000	135	1,103:1	0.09
2	0		12,200	170	71:1	1.39
	24	4.4	6,200	725	9:1	11.7
	24	15.5	4,600,000	660	6,969:1	0.014
3	0		17,300	140	123:1	0.809
	24	4.4	5,850	60	97:1	1.02
	24	15.5	335,000	75	4,466:1	0.022
4	0		885,000	1,100	804:1	0.124
	24	4.4	575,000	460	1,250:1	0.08
	24	15.5	3,950,000	620	6,370:1	0.015
5	0		5,900	7	842:1	0.118
	24	4.4	6,200	4	1,550:1	0.064
	24	15.5	26,000	7	3,714:1	0.027

produced under conditions not so satisfactory and with unsterile utensils. Bacterial plate counts were made of the fresh samples. Each sample was then divided into two portions. One of these portions was pasteurized immediately while the other was placed at the holding temperature used and held for twenty-four hours. At the end of this holding period, the storage portion was pasteurized. In every case plate counts were made immedi-

ately before and immediately after pasteurization. The results of five experiments in which storage temperatures of 40°F. (4.4°C.) and 60°F. (15.5°C.) were used are given in table 3.

A study of these data reveals clearly that the bacterial population of milk becomes more sensitive to pasteurization when held at 60°F. (15.5°C.). It will also be noted that a considerable increase in numbers of bacteria took place at this temperature. In other words, the bacterial population was made up of relatively young cells and was, therefore, less resistant to heat. On the other hand, in the samples held at 40°F. (4.4°C.) the plate counts indicate that no appreciable growth took place and in these cases the bacterial cells did not become more sensitive to heat.

DISCUSSION

From the observations recorded in this paper, the general conclusions seem justified that a higher percentage destruction of bacteria by pasteurization will occur in milks which have been held at temperatures which allow the multiplication of the bacteria contained therein, than in milks of the same original flora which have been stored at temperatures sufficiently low to inhibit bacterial growth. It is further shown that this difference in resistance to heat may be explained on the basis of the difference in ages of the bacterial cells involved. In this connection it should be recorded that the results reported in the foregoing tables have been verified by a number of other experiments which have not been tabulated in this paper.

The practical results obtained when milks are held at 40°F. (4.4°C.) and 60°F. (15.5°C.) are in perfect accord with what would be expected on the basis of present knowledge of bacterial physiology. The point might well be raised as to what behavior should be expected if milk has been stored at 50°F. (10°C.). From the works of numerous investigators, it is well known that milks frequently show little or no increase in bacterial numbers when held at this temperature for 24 hours, whereas, in other samples a significant increase in bacteria takes place. In other words, it would be expected that the relative destruction of bacteria by pasteurization in milks held at 50°F. (10°C.) would show

considerable irregularity. For these reasons we have not emphasized our results obtained upon milk held at this temperature. The obvious expectation would be to find in milks stored at 50°F. (10°C.) a variation in behavior, in some cases simulating the results obtained at 40°F. (4.4°C.), and at other times obtaining results comparable to those secured at 60°F. (15.5°C.), depending upon whether bacterial growth had occurred. This, in fact, was found to be the case. Another irregularity in milk held at 50°F. (10°C.) has been noted: In one case a sample of milk held at this temperature showed no measurable increase in plate count during the twenty-four-hour period, but the results after pasteurization indicated a marked increase in the susceptibility of the bacteria to pasteurization. This particular irregularity is to be expected from what is known of bacterial physiology. It is well known that old bacterial cells pass through a dormant, or "lag," period before multiplication begins. As has been shown by Sherman and Albus (7), during this period of dormancy, the old cells, just prior to the initiation of growth, assume the same physiological characteristics as young cells. It is, therefore, logical to expect that when milk is kept at a temperature of 50°F. (10°C.), where growth proceeds slowly, that we should occasionally experience a marked increase in susceptibility of the bacteria to heat without an increase in the plate count.

Other points of practical interest should be mentioned. It is again made clear by this work that the integrity of the pasteurization process cannot be judged on the basis of the percentage destruction of bacteria. Aside from the reasons here emphasized, there are good and sufficient reasons for discarding that view and it has been, in fact, long since discredited. Those interested in milk control problems are at the present time emphasizing bacterial limits on the raw milk previous to pasteurization. Our results show the soundness of such standards, since it is entirely possible to obtain pasteurized milks of similar bacterial content when the raw milks from which they were derived varied greatly in germ content, depending upon the temperatures of holding previous to pasteurization.

With respect to the qualitative efficiency of pasteurization,

the physiological age of the bacterial cells is of especial significance and is worthy of careful consideration by those interested in the practical aspects of pasteurization. It is shown, for example, that two different types of ropy milk organisms may be eliminated from milk by pasteurization when young cells are involved, while old cells of the same organisms may withstand the same heat treatment. It is perhaps pertinent to suggest that some consideration should be given to the relative thermal death rates of young and old cells of milk-borne pathogenic bacteria.

It hardly seems necessary to repeat that it is recognized that differences in the physiological ages of the bacterial cells is only one factor in the variations observed in the efficiency of pasteurization when applied to milks of varying quality and bacterial populations. Obviously, the bacterial flora of a sample of milk will not be the same with respect to relative numbers of different types present after extensive growth has occurred. Nevertheless, it is believed that the data presented establish the practical importance of the age of bacterial cells in the pasteurization of milk.

SUMMARY

The age of bacterial cells influences greatly the susceptibility of the organisms to heat, young cells being more easily killed than are older ones.

Milks which have been kept at temperatures at which bacterial growth takes place (e.g., 15.5°C.) show a greater percentage destruction of bacteria than occurs in similar milks when fresh or when held at temperatures (e.g., 4.4°C.) too low to allow multiplication of the bacteria involved.

It has been established that some important milk bacteria (e.g., "ropy milk" types) may be entirely eliminated from milk by pasteurization when the cells are in a young and growing condition, while the old cells of the same organisms are able to withstand the process.

A number of points of practical significance in connection with pasteurization are discussed.

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FURTHER STUDIES OF THE RÔLE OF VITAMIN C IN THE NUTRITION OF CALVES*

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A previous publication (1) has reported experiments showing that calves do not require vitamin C in their feed in quantities that can be measured by the present method of testing food materials for their antiscorbutic potency by feeding them to guinea pigs. Further results have been obtained which have no effect on the practical phase of this problem, but which have extended somewhat our knowledge of vitamin C in relation to the bovine. This paper is concerned with a study of the presence of vitamin C in the livers of two of the calves and in the milk of a heifer grown from birth on the vitamin C-deficient ration and with an attempt to locate the source of vitamin C supply to calves receiving rations deficient in this vitamin.

VITAMIN C TESTS OF LIVERS

After one year of experimental feeding as previously described (1) the livers of control calf E-44, and experimental calf E-45 were removed at autopsy and immediately frozen in a sharp freezer. The antiscorbutic value of these organs was then tested by experimental feeding of guinea pigs.

Ten guinea pigs of approximately 3000 grams weight were used in this experiment. To insure the health of the animals, especially as regards their freedom from scurvy, they were fed a diet of green grass and grain for a period of 6 days before experimental feeding was begun. The excellent condition of these animals was indicated by their glossy, smooth hair, bodies

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that were plump and vigorous, and by their gain in weight during this preliminary feeding.

In this experiment a basal ration of grain mixture and timothy and wild hay was furnished. No record of the consumption of this food was kept, because it has been found (2) (3) that inanition does not produce scurvy. The animals were observed to eat the basal diet well.

The preparation of the liver for feeding consisted of chipping pieces from the frozen organ, weighing the required quantity into a porcelain dish, and adding 8 or 10 cc. of water. No attempt was made to macerate the tissue. This preparation was allowed to stand at room temperature for approximately one-half hour, after which the tissue was removed and the water extract fed. This method of preparation was resorted to because a previous trial, in which the feeding of macerated liver was attempted, gave results which were unreliable and inconclusive. The water extract of liver was force-fed by means of a syringe. It was surprising to find that, after becoming accustomed to this food, several of the animals developed a liking for it and aided in its administration. No such observation was made when the tissue itself was fed.

Both the preventive and the curative methods of study were used in this work. Four of the 10 guinea pigs were fed the extract of 10 grams of liver throughout the entire period of experimental feeding. Two of these received the extract from E-44, while the other two received that from E-45. Four other guinea pigs were fed the basal ration until the development of scurvy, after which the curative powers of extracts of the two livers were tried. The two remaining animals were used to test the antiscorbutic value of concentrated extracts of the two livers.

For the preparation of the concentrated extracts 1000 grams of liver were allowed to stand for three hours in about 3 liters of distilled water acidified with hydrochloric acid. The tissue was then removed as completely as possible by straining through cheese cloth, after which the volume of the extract was reduced to about 500 cc. by evaporation under a 25-inch vacuum. By this means the temperature of the liquid never exceeded 65°C.

A thick brown liquid resulted, which was stored in a bottle, and covered with paraffin oil to avoid oxidation of the vitamin.

Two guinea pigs received 10 cc. of this preparation daily in addition to the basal ration. One of the animals fed this concentrated material died soon after experimental feeding was

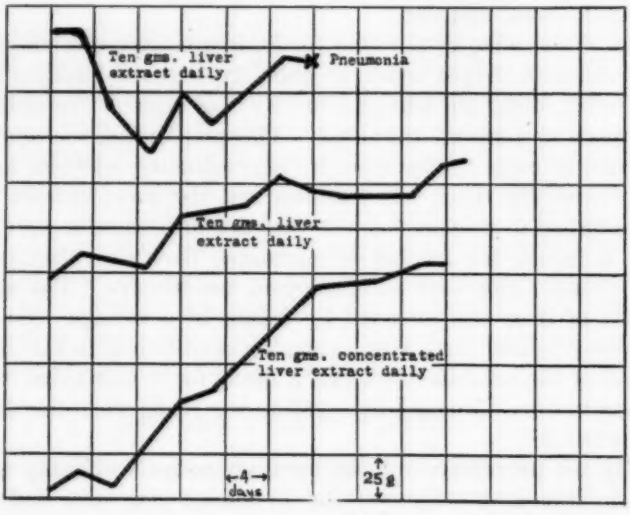

CHART 1. ANTISCORBUTIC VALUE OF LIVER OF E-44

Weight curves of guinea pigs fed extract of liver from control calf E-44. Ration, grain mixture, timothy and prairie hay ad libitum, and extract of liver. One guinea pig died of pneumonia after making gains for 15 days, but the other two remained healthy. The third guinea pig, fed a concentrated extract of the liver, made excellent gains, and showed every sign of excellent health at the close of the experiment. The antiscorbutic value of the liver here demonstrated was expected, because, previous to this test E-44 had been receiving 325 cc. of tomatoes daily for a year.

begun. Autopsy failed to reveal the cause of death but a toxicity of the preparation was blamed. No symptoms of scurvy were evident in this animal.

The results of the liver feeding experiment revealed considerable antiscorbutic value in the organs of both the experimental and control calves. Except for guinea pig 3, which died from

abscessed lungs, the animals fed the unconcentrated extract of frozen liver lived and gained in weight throughout the 41 days of experimental feeding. Cures of scurvy were difficult to bring about because, in most cases, the disease was too far advanced

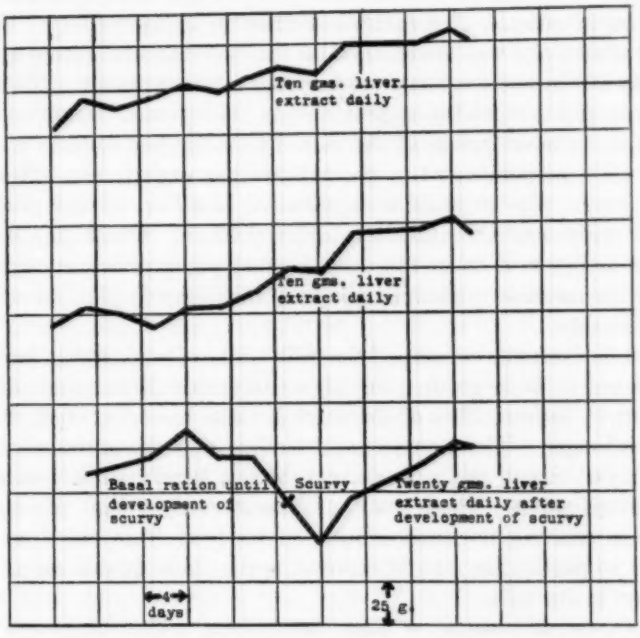
Ten gms. liver extract daily
Ten gms. liver extract daily
Basal ration until development of scurvy
Scurvy
Twenty gms. liver extract daily after development of scurvy
4 days
25 g.

CHART 2. ANTISCORBUTIC VALUE OF LIVER OF E-45

Weight curves of guinea pigs fed extract of the liver of experimental calf E-45. Ration, grain mixture, timothy and prairie hay ad libitum, extract of liver. Two of the guinea pigs showed good gains throughout the experiment and were healthy at the close. The third guinea pig was allowed to develop scurvy on the basal diet after which a cure was effected by the daily administration of the water extract from 20 grams of liver. E-45 had been fed the vitamin C-deficient ration for one year previous to this test.

before it was noticeable. However, one cure was effected. The one animal fed the concentrated product from the liver of the control calf, showed the best development of any of the experimental animals. At the close of the experiment it was the most vigorous of the entire experimental group.

The weight curves of those animals which lived long enough to give definite results are shown in charts 1 and 2.

Antiscorbutic value of milk of E-39. When E-39 calved she had been fed since birth on the scorbutic ration. For three months previous to her birth her dam had also received the scorbutic ration. Her calf was normal in every respect. After parturition she was maintained on the experimental ration and a study of the antiscorbutic value of her milk was made. Twenty guinea pigs, weighing approximately 250 grams, were fed, ad libitum, a basal ration of the mixed timothy and wild hay, and the grain mixture used in the calf-feeding experiment. Ten of the guinea pigs received a supplement of 30 cc. of the milk of E-39 daily, and the other ten received 60 cc. Other conditions were maintained as in the other guinea pig experiments except that the animals were weighed every third day in place of every second day.

All the animals consumed the milk well. Considerable growth occurred in both groups, but those receiving 30 cc. succumbed to scurvy in from 29 to 47 days, while those receiving 60 cc. lived much longer. The experimental feeding was discontinued after 83 days. Six of the animals had died of scurvy while four still remained alive. These results make it evident that when the bovine receives no antiscorbutic in its food from the time of birth to parturition, it still secretes appreciable quantities of the factor in its milk.

Antiscorbutic value of hay concentrate. The discovery of vitamin C in the liver of a calf fed for one year on a ration deficient or lacking in this factor leaves the question of the calf's requirement for vitamin C unsettled. The possibility that the bovine requires the factor, and that the ration supplied it in quantities too small to be measured by tests with guinea pigs immediately suggested itself. Grain has so often been found to be scorbutic that any study of it as a source of vitamin C seemed needless. Less work had been done with the dry hays, and an experiment was therefore planned to attempt to detect minute quantities of the antiscorbutic vitamin in the mixed timothy and wild hay from the lot fed the calves in experiment 2.

The futility of attempting to demonstrate antiscorbutic value in this day by direct feeding was emphasized by the previous test of the hay with guinea pigs. Concentration of the water-soluble constituents of the hay offered the most logical approach to the problem, and was the method adopted.

Thirty-six pounds of ground timothy and wild hay were placed in earthen jars and covered with distilled water, buffered by monobasic potassium phosphate to maintain an acid reaction. These suspensions were held for twenty-four hours at a temperature of approximately 4°C. The resulting brown extract was then removed and concentrated in vacuo to about one-fifth its original volume. About 2½ gallons of thick brown liquid resulted. This was treated with 2 gallons of absolute alcohol which produced a voluminous precipitate. After standing over night at 4°C., it was filtered, slightly acidified with acetic acid and evaporated under vacuum to a volume of about 1 liter. Following the procedure of Zilva (4), basic lead acetate was added until no more precipitate formed. The solution was then distinctly alkaline to litmus. According to the work of Zilva (4), as well as that of Bezssonov (5), this precipitate should contain vitamin C if any were present. The precipitate was repeatedly washed by suspending it in water containing basic lead acetate, allowing to settle and then decanting. This washing was found necessary because a previous trial had shown that some poisonous principle was held by the basic lead acetate precipitate, and that its removal could be effected by continued washing. After about six washings, the precipitate was suspended in a small quantity of acidified water and deleaded. A clear colorless liquid resulted which was made up to 500 cc. with distilled water and stored in glass at ice box temperature. Paraffin oil was placed over the surface of the preparation to prevent oxidation of any vitamin C present.

Five guinea pigs were fed a basal ration of timothy and wild hay, and the experimental grain mixture, with additions of 5 cc. of the concentrated preparation. Scurvy developed very quickly. It was assumed, therefore, that no trace of vitamin C was supplied the experimental calves in their hay.

Weight curves of the guinea pigs used in this experiment are shown in chart 3.

Antiscorbutic value of feces. The results of the foregoing experiments had practically eliminated the ration as a direct source of vitamin C to the experimental calves. It therefore became reasonably certain that the bovine is capable of producing

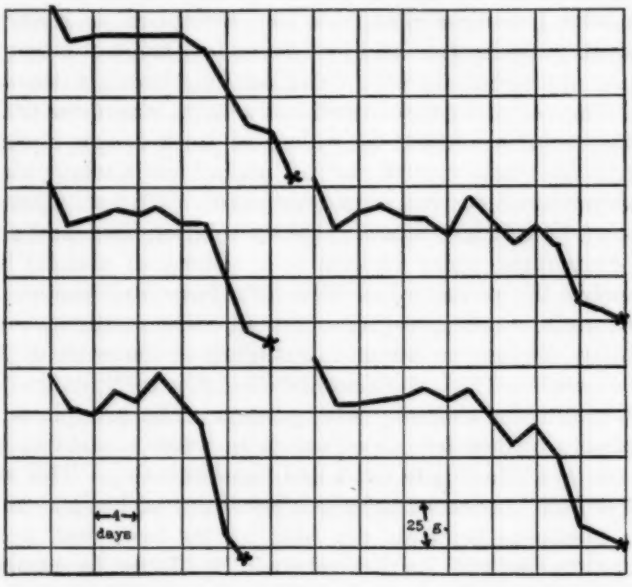

CHART 3. ANTISCORBUTIC VALUE OF CONCENTRATED EXTRACT OF TIMOTHY HAY

Weight curves of guinea pigs fed concentrated extract of timothy hay in addition to the basal diet. Ration, grain mixture, timothy and prairie hay ad libitum, 5 cc. concentrated extract. Extract equivalent to 0.36 pound of hay was fed daily but no protective action against scurvy was evident.

the antiscorbutic vitamin within its body. The possibility that the vitamin is produced at some point in the digestive tract was at once apparent. Fortunately, a test of the antiscorbutic value of the feces of calves fed the vitamin C-deficient ration had been made during the first experiment with calves.

Nine guinea pigs, weighing approximately 300 grams, were

used in this trial. They were fed a basal ration of grain and water. Feces were collected daily and fed fresh. As expected, the guinea pigs refused to eat the feces when fed separately, and it became necessary to mix them with the grain. A mixture

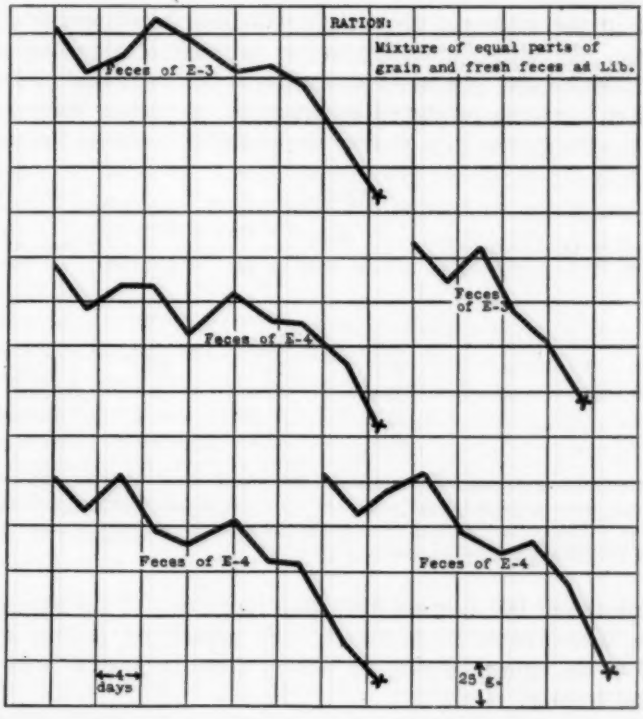

CHART 4. ANTISCORBUTIC VALUE OF FECES FROM EXPERIMENTAL CALVES

Weight curves of guinea pigs fed a mixture of equal parts of the grain mixture fed the experimental calves and fresh feces either from E-3 or E-4, both of which were fed the vitamin C-deficient diet. Vitamin C could not be demonstrated in the feces, for all the guinea pigs died of scurvy.

composed of equal parts by weight of grain and fresh feces was eaten with reluctance by the animals, but proved of no anti-scorbutic value. The animals died of scurvy in about 30 days. The weight curves were characteristic of those where scurvy develops quickly.

Table 1 shows the food consumption record for the animals used in this experiment. The weight curves are shown in chart 4.

Antiscorbutic value of stomach contents of calves fed the vitamin C deficient ration. The knowledge that vitamin C is produced in grain soaked in water for a few hours (6) led to the belief that grain in the stomachs of cattle might also be subject to this process. In studying this question an animal receiving the experimental diet of grain and dry hay was slaughtered and her stomach contents preserved by freezing. A test of these materials with guinea pigs proved unsuccessful, however, because

TABLE 1

NUMBER OF GUINEA PIG	DAYS ON EXPERIMENT	CALF STUDIED	DAILY FEED CONSUMPTION		CONDITION AT AUTOPSY
			Grain and feces	Feces (calculated)	
			grams	*grams*	
1	30	E-3	12.6	9.3	Scurvy
2	30	E-3	20.0	10.0	Scurvy
3	31	E-4	19.5	9.8	Scurvy
4	16	E-3	5.9	3.9	Pneumonia
5	27	E-4	29.5	14.8	Scurvy

Guinea-pigs weighing approximately 300 grams were used in this test. The feces were collected fresh each day and mixed with the grain in equal portions. The consumption of feces is therefore calculated on the basis that half of the feed consumed consisted of them.

all but one of the animals died from impaction of the stomach before scurvy could be produced. One guinea pig, fed the contents of the rumen, developed scurvy after 18 days of experimental feeding.

This result, although inconclusive, indicates that vitamin C is not produced within the rumen.

DISCUSSION

The results of these and our previous experiments indicate conclusively that the bovine does not require vitamin C in quantities that can be detected by tests with guinea pigs. Four calves grew at a normal rate or better for a period of one year, on a

ration which produced scurvy in guinea pigs in 20 to 30 days. Even when the possibility of vitamin C storage was minimized by maintaining the pregnant cow on a ration lacking this factor, the feeding of the resulting calf on a similar ration from the time of birth until one year of age failed to cause any perceptible ill-effects. Perfectly normal gestation and parturition occurred in a heifer fed from birth on a vitamin C-deficient ration. From the standpoint of the practical feeder it can be said that the bovine has no dietary requirement for vitamin C.

It cannot be said, however, that the bovine does not need vitamin C, for, even though fed for over a year on the scorbutic diet, E-39 produced milk which was normal, or nearly so, with respect to its content of vitamin C, and the factor was also found in the liver of one of the calves after a year of experimental feeding.

The results obtained in these experiments indicate that the animals did not obtain even minute quantities of vitamin C from their feed, and its synthesis within their bodies is indicated. How and where this synthesis takes place was not determined. The results from tests of the antiscorbutic value of feces and stomach contents of calves fed the scorbutic ration were all negative, and although somewhat inconclusive, due to insufficient data, all indicate that this synthesis occurs elsewhere than in the digestive tract.

The discovery of the mechanism by which the antiscorbutic vitamin is produced in the body of the calf would be of considerable technical value, because it should then be possible to determine whether or not vitamin C is required by the bovine. The similar problem presented in the rat and chicken then might also be explained. Based on the discoveries made in these experiments, the production of this vitamin by some body organ is indicated, and the enlargement of the adrenal glands in guinea pig scurvy suggests that it may be intimately concerned with this factor. Perhaps the adrenals of the bovine, rat and chicken are capable of synthesizing vitamin C, while failing of this function in man, the guinea pig and monkey.

CONCLUSIONS

1. Vitamin C can be demonstrated in the livers of calves fed for one year on a ration capable of producing scurvy in guinea pigs within 30 days.

2. Heifers fed from birth on a scorbutic diet secrete appreciable quantities of vitamin C in their milk.

3. The absence of vitamin C from the diet apparently does not interfere with reproduction in cattle.

4. Vitamin C is probably synthesized within the body of the bovine. Evidence is supplied to indicate that the digestive tract is not concerned in this synthesis.

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THE SOLUBILITY OF CALCIUM PHOSPHATE IN FRESH MILK*

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A knowledge of the calcium and phosphate equilibria in milk is fundamentally important, especially to investigators of nutritional and dairy manufacturing problems. No satisfactory method of determining calcium ion concentration in milk has been found and, largely for this reason, the state of the phosphate phosphorus has not been definitely determined.

Recent work by Holt, La Mer and Chown (1), by Hastings, Murray and Sendroy (2), by Sendroy and Hastings (3), and by Shear and Kramer (4) has done much toward clarifying the physico-chemical phase of the deposition of calcium salts in bone. The present article attempts to transfer to milk equilibria the results of these investigations on the behavior of the phosphate-calcium system, and to calculate therefrom the calcium ion concentration and the calcium-phosphate ion product of milk. It is realized that some of the assumptions made are open to criticism and it is hoped that both the assumptions and the calculations will be examined critically, but with constructive purpose.

A structure built on any assumption regarding the degree to which the calcium of milk is ionized is rather insecure on account of the presence in milk of considerable quantities of citrate and phosphate and on account of the comparatively large quantity of calcium itself. The assumption of complete ionization of calcium in blood serum, on the other hand, seems justified for mathematical purposes and has been made in the calculations of the investigators cited above. That, however, at least a part of the calcium of blood serum exists as a slightly, or entirely, un-ionized but diffusible compound has been indicated by work of Green-

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wald (5), Klinke (6), Sendroy and Hastings (3), and Shelling and Maslow (7). Shear and Kramer (4, Paper V) have shown by conductivity titration of calcium chloride solution "direct evidence for the binding of calcium ions by sodium citrate in some kind of soluble complex." This situation may be of consequence in the case of milk.

That the ion product $[Ca^{++}] \times [HPO_4^{--}]$ is the criterion for precipitation of calcium phosphate was the conclusion of Shear and Kramer (4, Paper III) from calcification experiments. From experimental data they derived the following expression for the negative logarithm of the corresponding solubility product: $pK_{s.p.}$ $CaHPO_4 = 6.5 - 2.6\sqrt{\mu}$ in which μ is the ionic strength, the sum of one-half the products of the molarity of each species of ion and the square of its valence. They claimed that the existence of a compound $Ca_3(PO_4)_2$ has never been demonstrated.

They found further that in the experiments of Holt, La Mer and Chown (1) and of Domontovitsch and Sarubina (8) "in which final equilibrium was attained by shaking precipitates of calcium phosphate with their supernatant solutions" a "constant" was obtained "when the analytically determined calcium concentration of the solution, expressed in mols per liter, was divided by the hydrogen ion concentration." This "constant" was found to increase with increasing ionic strength of the solution. The expression for this relationship was derived as:

$$\log \frac{[Ca^{++}]}{[H^+]} = 2.4 + 2.2\sqrt{\mu}$$

From the equation given above and from analytical data on milk serum has been calculated the calcium ion concentration, $[Ca^{++}]$, of milk at the hydrogen ion concentration of pH 6.6. In the first calculation of μ, $[Ca^{++}]$ was disregarded. The value for $[Ca^{++}]$ obtained in the calculation was then inserted in the data sheet (figures in parenthesis) and the whole calculation repeated in order that the final value might be consistent with that used for calculation of ionic strength. The second value checked with the first within the limits of proportional accuracy implied by the equation. The analytical data used were those

of Van Slyke and Bosworth (9) on milk serum. The total citric acid was reduced by an amount equivalent to the serum calcium on the assumption that this amount of citric acid was removed from

TABLE 1

The ionic strength of normal fresh milk serum. pH = 6.6

	DISSOCIATION CONSTANT	CONCENTRATION		IONIC STRENGTH, ½ (MOLARITY) (VALENCE)²
		grams per liter	*mols per liter*	
K^+		1.24	0.0318	0.0159
Na^+		0.57	0.0249	0.0125
Mg^{++}		0.08	0.0033	0.0066
Ca(milk)		1.27	0.0318	
Ca(serum)		0.465	0.0116	
Ca^{++}			(0.00028)	(0.0006)
H^+			0.0_63	
OH^-			0.0_54	
Cl^-		0.81	0.0229	0.0115
P_2O_5		1.42	0.200[P]	
$H_2PO_4^-$	1×10^{-2}		0.0143	0.0072
$HPO_4^=$	1×10^{-7}		0.0057	0.0114
$PO_4^{\equiv}$	4×10^{-13}		0.0_99	
H_3Cit (total)		2.37	0.0123	
H_3Cit (residual)			0.0047	
H_2Cit^-	1×10^{-3}		0.0_52	
$HCit^=$	4×10^{-5}		0.0004	0.0008
$Cit^{\equiv}$	3×10^{-6}		0.0043	0.0194
				$\Sigma\mu = 0.0853$ (0.0859)

$\sqrt{\mu} = 0.2921$ (0.2931)

$$\log \frac{[Ca^{++}]}{[H^+]} = 2.4 + 2.2\sqrt{\mu} = 3.04$$

$$\frac{[Ca^{++}]}{[H^+]} = 1096. \quad [Ca^{++}] = (1096)(0.251 \times 10^{-6}) = 0.00028$$

$$[Ca^{++}] \times [HPO_4^=] = (0.00028)(0.0057) = 0.160 \times 10^{-6}$$

$$pK_{s.p.}\ CaHPO_4 = 6.5 - 2.6\sqrt{\mu} = 5.74. \quad K_{s.p.} = 0.182 \times 10^{-6}$$

the ionic condition as a calcium citrate complex in accordance with the ideas quoted above. The remaining "residual" citric acid was made the basis for the calculation of the contribution of the citric acid to the ionic strength. The equations derived by

Holt, La Mer and Chown (1) were used for calculating the distribution of the phosphoric and citric acids among the three ionic forms of each. The results of these calculations are shown in table 1.

The value 0.00028M for calcium ion concentration obtained by this procedure represents $\frac{0.00028}{0.0318}$, or approximately 1 per cent of the total calcium of milk. Of the diffusible calcium of milk $\frac{0.00028}{0.0118}$, or 2.5 per cent, appears by calculation to be ionized. In other words, although approximately 36 per cent of the total calcium is diffusible, apparently only one part out of each diffusible 36 parts is ionized.

An attempt was made to test experimentally the validity of these calculations. In each of two 100 cc. graduates was placed a solution containing 0.0465 gram Ca as $CaCl_2$. To the contents of one was added 0.2370 gram citric acid previously dissolved and adjusted to about pH 7.0 with sodium hydroxide solution. To each was then added a solution of disodium phosphate containing the equivalent of 0.142 gram P_2O_5. Both were adjusted to the same hydrogen ion concentration, approximately pH 6.6, and immediately made up to 100 cc. with distilled water. Precipitation took place in the flask which contained no citrate; the contents of the flask to which citrate had been added became slightly opalescent, but precipitation did not occur. These results are taken to indicate that the presence of citrate in milk prevents the precipitation of calcium phosphate, an effect probably due to the previously discussed de-ionizing action of citrate on calcium which reduces the calcium ion concentration below that requisite to the solubility product of dicalcium phosphate.

SUMMARY

A calculation has been made of the calcium ion concentration of normal fresh milk. This calculation is based on recent experimental results bearing on the mutual effects of hydrogen, calcium, phosphate and citrate ions on one another.

A calculation of the ion product $[Ca^{++}] \times [HPO_4^{=}]$ in normal fresh milk gives a value less than that of the corresponding solubility product.

Experimental evidence as to the validity of the method of calculation is offered in the form of an experiment in which the precipitation of calcium phosphate was prevented by the presence of citrate.

It is concluded that the solubility of calcium phosphate in milk is affected by the amount of citrates present and that this effect is probably specific.

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EFFECT OF COTTONSEED MEAL AND LINSEED OIL MEAL ON THE CONSISTENCY OF FECES OF DAIRY CATTLE*

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It is the common opinion of investigators and feeders of livestock that cottonseed meal is costive and linseed oil meal is laxative when fed to dairy cattle. According to Henry and Morrison (1), cottonseed meal is constipating, and should be fed with laxative concentrates such as linseed oil meal, wheat bran, or with succulent feeds such as silage or root crops. McCandlish (2) stated that cottonseed meal has a constipating effect and should be fed with laxative feeds such as linseed oil meal and wheat bran. He also stated that cottonseed meal is an excellent feed for cows on pasture. According to Eckles (3), cottonseed meal is somewhat constipating. Bull (4) stated that cottonseed meal has a costive action.

However, Ewing and Wells (5) observed that cottonseed meal in small quantities produces a normal feces, but, when it was the sole ingredient of the ration, the bowel action became slightly laxative. Wright (6) noticed that cattle fed a ration of 8 pounds of cottonseed meal and 25 pounds of cottonseed hulls with a feed of hay once a week excreted soft feces. The feces were made normal by increasing the hay in the ration. Reed, Huffman and Addington (7) reported that there was but very little difference between the rate of food passage through the digestive tract when cottonseed meal and linseed meal were fed. Curtis (8) stated that cottonseed meal is laxative when fed to horses.

The purpose of this investigation was to determine the effect of feeding cottonseed meal and linseed meal on the consistency of feces of dairy cattle.

* Received for publication January 4, 1929.

EXPERIMENTAL

Method used

The consistency of different feces has usually been measured by observing the physical appearance. In this investigation, the following mechanical method was used. The feces samples were taken immediately after evacuation. A 6 inch evaporating dish was filled with the fresh feces and leveled off with a smooth stick. A wooden ball with graduations upon its circumference was gently placed in the center of the feces in the evaporating dish.

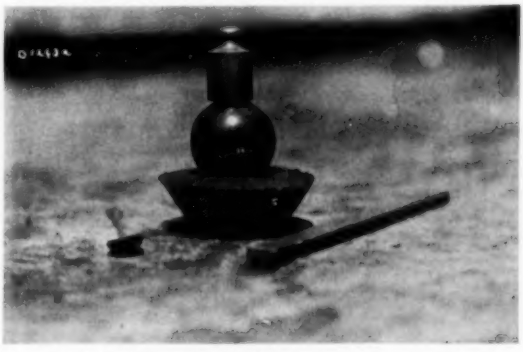

FIG. 1. SHOWING MECHANICAL METHOD USED IN DETERMINING CONSISTENCY OF FECES

Each graduation was called a degree. These graduations read from 15° to 45°. A 500-gram weight was placed upon the top of the ball which was flattened on top. The distance which the ball indented the feces was determined by the graduations. Hence, the softer the feces the further the ball sank and the greater the reading in degrees and vice versa. The determinations were made about the same time each day.

PART I

The effect on the consistency of the feces of cottonseed meal and linseed oil meal when fed with timothy hay and corn silage

The consistency of feces of six Holstein heifers which were from 24 to 30 months of age was determined. These animals

TABLE 1

Showing the effect on the consistency of the feces of cottonseed meal and linseed oil meal when fed with timothy hay and corn silage

ANIMAL NUMBER	NUMBER OF DEGREES WHICH BALL SANK IN FECES										RATION				
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	Cotton-seed meal	Linseed oil meal	Corn	Silage	Timo-thy
Lot I															
											pounds	*pounds*	*pounds*	*pounds*	*pounds*
G 1	22	21	20	21	28	20	27	23	29	24	7.8		2	25	14
G 3	35	30	25	30	30	36	35	25	34	32	9.0		3	25	15
G 5	40	25	28	26	28	29	30	30	36	30	9.0		3.9	25	12
Daily total	97	76	73	77	86	85	92	78	99	86					
Daily average	32.3	25.3	24.3	25.7	28.3	28.3	30.7	26.0	33.0	28.7					
Lot II															
G 2	29	25	25	30	23	25	25	30	36	25		8.2		25	13
G 4	27	22	25	25	25	32	25	25	35	32		12.6	3	14	13
G 6	23	20	20	21	28	32	25	26	20	32		6.0	3	18	12
Daily total	79	67	70	76	76	89	75	81	91	89					
Daily average	26.3	22.3	23.3	25.3	25.3	29.7	25.0	27.0	30.3	29.7					

Grand total of degrees, lot I, cottonseed meal..... 849
Grand total of degrees, lot II, linseed oil meal..... 793
Difference: Cottonseed meal feces 56 degrees softer than linseed oil meal feces

are being used on a long time experiment to determine the effects on health and reproduction of heavy cottonseed meal and linseed oil meal feeding. Three animals in lot I, G 1, G 3, and G 5 have received cottonseed meal as the principal source of protein, and three animals in lot II, G 2, G 4, and G 6, have received linseed oil meal as the principal source of protein since they were 90 days of age. These observations were taken during heavy milk production. The effect of these feeds on the consistency of feces is shown in table 1. The results show very little difference in the consistency of feces of the two groups even though the cottonseed meal heifers received from 7.8 to 9.0 pounds of cottonseed meal daily and the linseed meal group received from 6.0 to 12.6 pounds of linseed oil meal daily.

PART II

The effect on the consistency of feces of cottonseed meal and linseed oil meal when fed with timothy hay and without corn silage

The results secured in part I indicated that there was practically no difference in the consistency of feces of cattle fed heavily on cottonseed meal or linseed oil meal along with silage and timothy hay. Since succulent feed, such as silage, is regarded as having a laxative effect, six mature cows were placed on an experiment to determine the effect of feeding large amounts of cottonseed meal and linseed oil meal without silage.

The cows were divided into two lots of 3 animals each. The cows in both groups received 4.5 pounds ground corn, 4.5 pounds ground oats each, and all the timothy hay which they would clean up in addition to the protein concentrate.

The animals in lot I, numbers 158, 179, and 150, received in addition, 9 pounds of cottonseed meal daily, while those in lot II, numbers 225, 226, and 217, received 9 pounds each of linseed oil meal daily. A seven day preliminary period was allowed, during which time the silage was replaced by timothy hay. The consistency of the feces excreted by the two groups was determined daily for 6 days, after which the animals in lot I were fed 9 pounds of linseed oil meal and those in lot II were fed 9 pounds of cottonseed meal daily. Two days were allowed for the change

TABLE 2

Showing the effect on the consistency of feces; of cottonseed meal and linseed oil meal when fed with timothy hay without corn silage

ANIMAL NUMBER	NUMBER OF DEGREES WHICH BALL SANK IN FECES														
	First Period						Change		Second Period						TIMOTHY
	Ration: 9 pounds cottonseed meal, 9 pounds corn and oats								Ration: 9 pounds linseed oil meal, 9 pounds corn and oats						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	
Lot I															
															pounds
155	39	40	38	36	37	30	36	39	38	32	34	41	31	40	22
150	37	35	28	37	40	31	30	31	32	36	34	30	23	31	18
179	44	34	34	38	35	33	31	28	33	36	35	38	30	38	22
Daily total.....	120	109	100	111	112	94	97	98	103	104	103	109	84	109	
Daily average.....	40.0	36.3	33.3	37.0	37.3	31.3	32.3	32.7	34.3	34.7	34.3	36.3	28.0	36.3	
Lot II															
	Ration: 9 pounds linseed oil meal, 9 pounds corn and oats						Change		Ration: 9 pounds cottonseed meal, 9 pounds corn and oats						
217	31	33	36	34	26	23	31	33	28	25	24	30	31	35	18
225	39	32	32	27	33	30	28	31	30	32	35	27	27	34	16
226	34	32	25	28	31	30	27	31	28	28	26	26	28	30	13
Daily total.....	104	97	93	89	90	83	86	95	86	85	85	83	86	99	
Daily average.....	34.7	32.3	31.0	29.7	30.0	27.7	28.7	31.7	28.7	28.3	28.3	27.7	28.7	33.0	

Sum total of degrees, lot I, 1st period, cottonseed meal... 646
Sum total of degrees, lot II, 2nd period, cottonseed meal... 524
Grand total of degrees for cottonseed meal.....1170

Sum total of degrees, lot I, 2nd period, linseed oil meal. 612
Sum total of degrees, lot II, 1st period, linseed oil meal. 556
Grand total of degrees for linseed oil meal.....1168

Difference: Cottonseed meal feces 2 degrees softer than linseed oil meal feces

from cottonseed meal to linseed oil meal and vice versa. The consistency of the feces from both groups was then observed daily for 6 days.

The effect of these feeds on the consistency of the feces is shown in table 2. It is apparent that there is but little difference between the consistency of feces of animals fed cottonseed meal and linseed oil meal. It is also of interest to note in tables 2 and 3 that the change from cottonseed meal to linseed oil meal and vice versa failed to produce a disturbance in the digestive tract. These animals were not accustomed to having cottonseed meal in their ration.

PART III

The effect on the consistency of feces of only two pounds of cottonseed meal and linseed oil meal when fed with timothy hay as a roughage

The results obtained in parts I and II indicated that the heavy feeding of cottonseed meal did not affect the consistency of feces any more than the heavy feeding of linseed oil meal. However, there was a possibility that the heavy feeding of cottonseed meal caused an irritation in the digestive tract which resulted in a soft feces. In order to determine the effect on the consistency of feces of feeding a small amount of cottonseed meal and linseed oil meal, 6 animals were placed on experiment.

The cattle in lot I, numbers 202, 194, and 1 B were fed 2 pounds of cottonseed meal, 3 pounds of corn and oats daily, with timothy hay for roughage. The cattle in lot II, numbers 153, 173, and 2B, received 2 pounds of linseed oil meal in addition to 3 pounds of corn and oats daily with timothy hay.

A seven day preliminary feeding period was allowed, after which, the consistency of feces was determined daily for eight days. Animals in lot I were then fed 2 pounds of linseed oil meal in place of cottonseed meal, and those in lot II were fed 2 pounds of cottonseed meal in place of 2 pounds of linseed oil meal. These changes were made in one day. Observations were made daily on the feces for eight days thereafter. The results are shown in table 3.

TABLE 3

Showing the effect on the consistency of feces of only two pounds of cottonseed meal and linseed oil meal when fed with timothy hay as a roughage

ANIMAL NUMBER	NUMBER OF DEGREES WHICH BALL SANK IN FECES																TIMOTHY
	First period								Change	Second period							
	Ration: 2 pounds cottonseed meal, 4 pounds corn and oats									Ration: 2 pounds linseed oil meal, 4 pounds corn and oats							
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day
Lot I																	
																	pounds
1 B	35	25	38	36	33	20	20	25	35	25	25	21	21	20	21	28	25	14
202	20	15	20	17	16	15	15	20	15	15	20	20	20	20	25	20	25	20
194	28	25	26	33	30	35	22	25	27	20	21	25	28	25	24	30	30	20
Daily total	83	65	84	86	79	70	57	70	77	60	66	66	69	65	70	78	80	
Daily averages	27.7	21.7	28.0	28.7	26.3	23.3	19.0	23.3	25.7	20.0	22.0	22.0	23.0	21.7	23.3	26.0	26.7	
Lot II																	
	Ration: 2 pounds linseed oil meal, 4 pounds corn and oats								Change	Ration: 2 pounds cottonseed meal, 4 pounds corn and oats							
2 B	36	26	27	31	30	25	26	25	27	32	25	25	25	25	27	28	25	14
153	35	38	35	35	35	33	38	35	38	43	35	38	30	30	37	35	38	22
173	31	30	36	43	30	30	28	30	30	23	21	28	30	25	33	30	35	20
Daily total	102	94	98	109	95	88	92	90	95	98	81	91	85	80	97	93	98	
Daily average	34.0	31.3	32.7	36.3	31.7	29.3	30.7	30.0	31.7	32.7	27.0	30.3	28.3	26.7	32.3	31.0	32.7	

Sum total of degrees, lot I, 1st period, cottonseed meal..... 594

Sum total of degrees, lot II, 2nd period, cottonseed meal..... 723

Grand total of degrees for cottonseed meal.....1317

Sum total of degrees, lot I, 2nd period, linseed oil meal..... 554

Sum total of degrees, lot II, 1st period, linseed oil meal..... 768

Grand total of degrees for linseed oil meal.....1322

Difference: Linseed oil meal feces 5 degrees softer than cottonseed meal feces

DISCUSSION

The method of judging the consistency of feces by physical appearance depends largely on the judgment of the investigator. The use of a wooden ball with graduations to measure the depth to which the ball sinks into different feces when a 500-gram weight is placed on it is a superior method of measuring the consistency of various feces.

Heifers which were raised on cottonseed meal as the principal source of protein from three months of age have excreted, for more than two years, feces which are just as soft as those excreted by animals raised on linseed oil meal for the same period. In this investigation, the amount of cottonseed meal or linseed oil meal did not affect the consistency of the feces.

When silage was left out of the ration in part II and cottonseed meal and linseed oil meal were fed at the rate of 9 pounds per day, no difference could be shown in the consistency of the feces. A sudden change from cottonseed meal to linseed oil meal feeding, and vice versa, had no apparent effect on the consistency of the feces. Cows which were fed only 2 pounds of cottonseed meal or 2 pounds of linseed oil meal daily excreted feces of about the same consistency. Apparently, the levels at which cottonseed meal was fed in these rations had no effect on the consistency of feces. The results support the findings of Ewing (5), Wright (6), Reed, Huffman and Addington (7), and Curtis (8) who observed no costive effect from feeding cottonseed meal.

SUMMARY

The results of this investigation indicate that cottonseed meal is not a costive feed for dairy cattle.

The consistency of feces of dairy cows is not altered by changing rapidly from a heavy cottonseed meal ration to a heavy linseed oil meal ration.

There was no appreciable difference between the effects of feeding a large amount and a small amount of cottonseed meal on the consistency of feces.

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BOOK REVIEWS

Dairy Bacteriology. BERNARD W. HAMMER, Professor of Dairy Bacteriology, Iowa State College. Published by John Wiley and Sons, Inc., New York, 1928.

The importance of microörganisms in the dairy is not appreciated as much as it should be by many who are engaged in the distribution of milk and in the manufacture of the various dairy products. One reason for this lack of appreciation has been the absence of any book which presents in an adequate manner the part which the various microorganisms play in the dairy. The book concerning which this note is being written will remove all reason for such unacquaintance with the rôles of microörganisms in milk and its products, since it presents in a clear, attractive, and adequate manner the general facts concerning the relation of microörganisms to the dairy. In its 473 pages one will find presented practically every subject in the field of dairy bacteriology, and in such a manner as to be understandable, not only to the student of dairying, but to those who are actually engaged in dairy manufacturing and who may have had little acquaintance previously with the subject of dairy bacteriology.

The general subject of milk fermentation is presented, together with the characteristics of the bacteria producing the various more important changes, such as the lactic acid fermentation, the ropy milk fermentation, and the gassy fermentation. The relation of bacteria to the spoilage of milk is presented in four chapters, one on contamination, one on reducing the contamination of milk and cream, another on the growth of microörganisms in milk and cream, and the fourth on the preservation of these products, in which is presented a complete discussion of pasteurization.

Approximately one-fourth of the book is devoted to the general subject of the spread of diseases by milk and its derivatives. The subjects of most immediate importance in this connection are Malta fever and contagious abortion; udder inflammations and septic sore throat.

The bacteriology of evaporated, sweetened condensed, and powdered milk, of ice cream, and of fermented milk is presented in three chapters.

The author has done much original work on the study of the bacteriology of butter cultures and of butter. One would therefore expect the presentation of this subject to be most adequate in the 65 pages devoted

thereto. The book ends with a chapter devoted to the bacteriology of cheese and another in which are described the tests for quality of milk and cream. Included in this discussion is a presentation of the limitations of the various tests commonly used in the manufacturing plant.

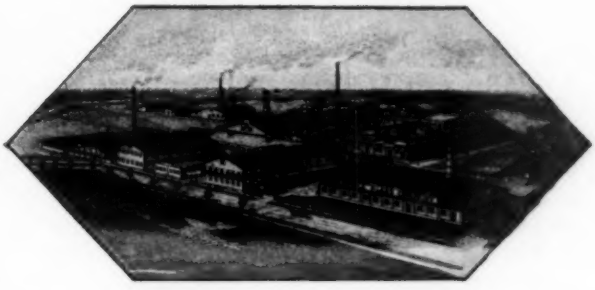
The book presents a very attractive appearance, both as to its exterior and interior.

The production of milk, its handling, its distribution, and its manufacture into the various products, involve an immense amount of bacteriology. The work will be done with the greatest success only as those engaged therein give conscious consideration to the bacteriology of the raw material and of the different manufactured products.

This book should find a place on the desk of everyone who is interested in the management of any phase of dairying. It is to be hoped that the demand for the book will justify frequent revision, in order that the subject may be kept abreast of the rather rapid advances in many of its phases.

E. G. HASTINGS.

K



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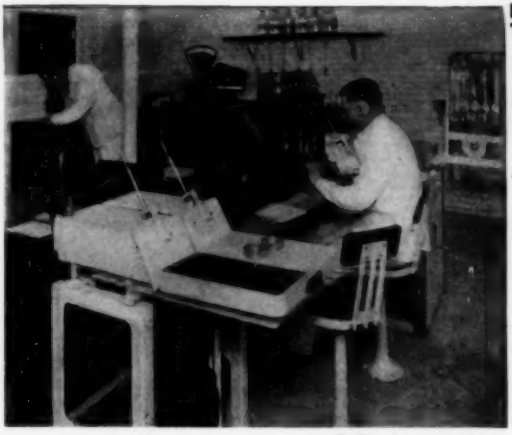

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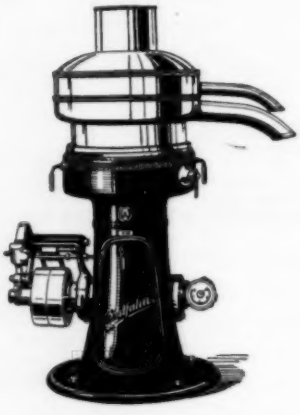

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